PS IN THE (CHANNEL) POD ARE NOT ALIKE. . .

Bidirectional Activity-Dependent Regulation of Neuronal Ion Channel Phosphorylation. Misonou H, Menegola M, Mohapatra DP, Guy LK, Park KS, Trimmer JS. *J Neurosci* 2006;26(52):13505–13514. Activity-dependent dephosphorylation of neuronal Kv2.1 channels yields hyperpolarizing shifts in their voltage-dependent activation and homoeostatic suppression of neuronal excitability. We recently identified 16 phosphorylation sites that modulate Kv2.1 function. Here, we show that in mammalian neurons, compared with other regulated sites, such as serine (S)563, phosphorylation at S603 is supersensitive to calcineurin-mediated dephosphorylation in response to kainate-induced seizures *in vivo*, and brief glutamate stimulation of cultured hippocampal neurons. *In vitro* calcineurin digestion shows that supersensitivity of S603 dephosphorylation is an inherent property of Kv2.1. Conversely, suppression of neuronal activity by anesthetic *in vivo* causes hyperphosphorylation at S603 but not S563. Distinct regulation of individual phosphorylation sites allows for graded and bidirectional homeostatic regulation of Kv2.1 function. S603 phosphorylation represents a sensitive bidirectional biosensor of neuronal activity.

COMMENTARY

Excitability governs the relationship between neuronal input and output. Therefore, its tight regulation is critically important for proper neuronal and circuit function. There are two contrasting elements to the optimal regulation of excitability: on one hand, the system should provide enough plasticity to enable it to adapt to constantly changing environments and demands; on the other hand, the system should be constrained within limits that ascertain stability and prevent extreme hyper- or hypoexcitability. Seizures are a clear example of the failure of stabilization mechanisms that are intended to prevent runaway network hyperexcitability. Therefore, uncovering the discrete processes by which neuronal excitability is constrained is important both for an understanding of seizure generation as well as for devising new ways to treat and prevent pathological conditions characterized by seizures, that is, various types of epilepsy.

Stability of neuronal excitability can be attained through processes such as homeostatic scaling of synaptic activity or through mechanisms influencing the intrinsic excitability of a neuron (1). In view of their pivotal role in the determination of excitability, ion channels constitute a key element of regulation of intrinsic neuronal excitability (1). Particularly suitable for this function are ion channels that are active in subthreshold membrane potentials (2); these include the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (3,4), T-type calcium channels (5), and members of the potassium channel family, such as the Kv2.1 delayed-rectifier (6), A-type, and "leak"-type (1,2).

Modulation of ion-channel activity can take place at several levels. For example, whereas transcriptional regulation can influence the number and identity of neuronal ion channels, already translated channels can be regulated by alterations in trafficking, membrane insertion, and internalization, thus controlling their subcellular distribution and surface expression. A more direct type of regulation can occur by modification of surface-expressed channels, which alters their biophysical properties (e.g., through phosphorylation or interaction with accessory proteins). The mechanisms mentioned not only target different aspects of channel regulation but function at different timescales. For instance, transcriptional regulation influences excitability starting within hours of the inciting stimulus and persisting for as long as a lifetime. Posttranslational mechanisms and channel modification, in particular, can occur within seconds and are often of shorter duration than transcriptional changes.

In the paper discussed here, Misonou and colleagues build on previous work and dissect in exquisite detail regulation of the Kv2.1 channel phosphorylation state as a mechanism of activity-induced reduction of neuronal excitability. The Kv2.1 channels are major contributors to the delayed rectifier potassium current at somatodendritic domains of cortical and hippocampal pyramidal neurons (6). The delayed rectifier potassium current regulates excitability at subthreshold potentials, and its activation results in subsequent hyperpolarization and suppression of neuronal activity (7,8). Specifically, Kv2.1 channels contribute to suppression of neuronal activity in response to high-frequency, repetitive input (6,7).

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Previously, the authors demonstrated a rapid activity-dependent alteration of expression pattern of these channels. Seizure induction in vivo as well as activity enhancement in vitro markedly shifted the channel expression from a clustered pattern to a diffuse one within minutes (9). A similar shift was observed in response to ischemia (8), and in both cases, the underlying mechanism involved elevations of intracellular calcium levels and dephosphorylation of the Kv2.1 channel through a calcineurin-dependent mechanism. It is important to note that these changes in channel expression pattern were coupled to alterations in Kv2.1 channel biophysical properties: the substantial hyperpolarizing shift (>20 mV) of the activation-curve, should increase the channel activity in subthreshold potentials and thus suppress neuronal excitability (8,9). Changes in the expression pattern of Kv2.1 channels were reversible, which was demonstrated by the fact that when excitatory input onto the neurons ceased, there was a gradual recovery of the clustering.

Misonou and colleagues identified the biochemical changes (phosphorylation) of the Kv2.1 channel molecules that mediated the activity-dependent changes in channel clustering and function. They used antibodies that discriminate among different phosphorylated residues in the Kv2.1 molecule and found two phosphorylation sites, serines 563 and 603 (S563 and S603), that are particularly sensitive to activity. Although these two sites are normally in a phosphorylated state, they undergo marked calcineurin-dependent dephosphorylation in response to in vivo kainate-induced seizures and ischemia as well as in response to in vitro glutamate-induced activation. Interestingly, the authors found distinct differences in the regulation of the two sites: the rate and magnitude of the S603 residue dephosphorylation were higher compared with those of the S563. Thus, these phosphorylation sites (Ps) within the Kv2.1 channel molecule are not similar in their response to activity, and differential regulation of the two sites might serve as a delicate sensor that accounts for the graded response of the channel to increased and reduced activity. Finally, the authors propose that this activity-dependent regulation is bidirectional, based on the fact that they demonstrated hyperphosphorylation of the S603 residue in response to suppression of activity by pentobarbital. Taken together, these studies delineate a homeostatic, graded mechanism—namely, bidirectional modulation of the Kv2.1 channel phosphorylation state—that may enable dynamic fine-tuning of intrinsic neuronal excitability in response to altered activity.

The elucidation of the molecular substrates of the mechanism involved in the modulation of the Kv2.1 channel contributes significantly to the understanding of how intrinsic neuronal excitability can be regulated and restricted in the normal brain. It also raises the possibility of failure of this stabilization mechanism within neuronal populations in the epileptic brain. The potential therapeutic value of activity-dependent regulation of KV2.1 channels is intriguing: if technically feasible, augmentation of selective Kv2.1 channel dephosphorylation might help stabilize neuronal excitability and, thus, may serve as a novel therapy for seizures.

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